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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

NATURAL AND ACQUIRED RESISTANCE IN THE BOVINE. EXPERIMENTAL PARASITOLOGY 51,000-000. A TOTAL OF 12 ANIMALS OF VARIOUS AGES WERE INFECTED WITH TRYPANOSOMA CONGOLENSE TO INVESTIGATE AGE RESISTANCE. TEN OF ELEVEN ANIMALS BETWEEN 4 MONTHS AND 1 YEAR OF AGE SURVIVED THE INFECTION WITHOUT TREATMENT. TWO OF ELEVEN ANIMALS IN THE AGE RANGE OF 1 TO 2 YEARS ALSO SURVIVED THE INFECTION WHEREAS ALL 20 ANIMALS BETWEEN 2 AND 5 YEARS OF AGE DIED OR NEEDED TREATMENT TO SURVIVE. YOUNG ANIMALS WHICH NEEDED NO TREATMENT TO SURVIVE WERE REFRACTIVE TO CHALLENGE FOR AT LEAST 1 YEAR AFTER THEIR LAST PATENT PARASITEMIA. OLDER ANIMALS

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WHICH REQUIRED TREATMENT TO SURVIVE WERE ALSO CHALLENGED AT INTERVALS AFTER THERAPY. THREE ANIMALS INFECTED FOR 49 TO 75 DAYS BEFORE TREATMENT WERE RE-CHALLENGED 198 TO 296 DAYS LATER. EXTENSIONS IN PREPATENT PERIODS RANGED FROM 5 TO 13 DAYS WHEN COMPARED TO CONTROLS AND THE RESULTING INFECTIONS WERE OF A RELAPSING NATURE FOLLOWED BY SELF-CURE. EFFECTS OF THIS DISEASE ON CLINICAL PARAMETERS OF PREVIOUSLY INFECTED ANIMALS WERE MINIMAL. ONE ANIMAL INFECTED FOR 196 DAYS AND RECHALLENGED 501 DAYS LATER HAD A PREPATENT PERIOD OF 14 DAYS AS COMPARED TO 5 DAYS FOR CONTROLS. THIS ANIMAL DEVELOPED A BRIEF RELAPSING IN-FECTION FOLLOWED BY SELF-CURE. ANIMALS WHICH WERE INFECTED FOR PERIODS 41 TO 77 DAYS, RECEIVED TREATMENT, AND WERE THEN RECHALLENGED FROM 600 TO 900 DAYS LATER, SHOWED SOME RESISTANCE TO INFECTION. PREPATENT PERIODS WERE EXTENDED FROM 1 TO 3 DAYS OVER THOSE OF CONTROL ANIMALS AND ALTHOUGH THE RESULTING DISEASE WAS SEVERE, ONE OF FOUR ANIMALS SELF-CURED WITHOUT TREATMENT. WHEN ANIMALS WHICH HAD SELF-CURED PRIMARY CHALLENGES WERE RECHALLENGED AT PERIODS UP TO 2 YEARS LATER, THEY WERE COMPLETELY REFRACTORY. WHEN 12 ANIMALS WHICH WERE PRESUMED TO BE IMMUNE TO SYRINGE-PASSAGED T. CONGOLENSE WERE CHALLENGED BY TSETSE FLY BITE WITH THE SAME STRAIN OF TRYPANOSOME, AN APPRECIABLE IMMUNITY WAS EVIDENT. FIVE OF TWELVE IMMUNE ANIMALS DID NOT BECOME PATENT WHILE THE OTHER SEVEN DEVELOPED MILD INFECTIONS WITHOUT SEVERE CLINICAL SIGNS. ALL NINE CONTROLS DEVELOPED SEVERE INFECTIONS WITH EIGHT REQUIRING TREATMENT TO SURVIVE. WHEN ANIMALS IMMUNE TO THE TRANS-MARA I STRAIN OF T. CONGOLENSE WERE CHALLENGED EITHER BY SYRINGE OR TSETSE FLY BITE WITH A HETEROLOGOUS STRAIN OF T. CONGOLENSE OBTAINED FROM A DIFFERENT GEOGRAPHICAL AREA, NO EVIDENCE OF IMMUNITY WAS DETECTED.

EXPERIMENTAL PARASITOLOGY 52, 219–232 (1981)

Trypanosoma congolense: Natural and Acquired Resistance in the Bovine

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WELLDE, B. T., HOCKMEYER, W. T., KOVATCH, R. M., BHOGAL, M. S., AND DIGGS, C. L. 1981. *Trypanosoma congolense:* Natural and acquired resistance in the bovine. *Experimental Parasitology* 52, 219–232. A total of 42 animals of various ages were infected with *Trypanosoma congolense* to investigate age resistance. Ten of eleven animals between 4 months and 1 year of age survived the infection without treatment. Two of eleven animals in the age range of 1 to 2 years also survived the infection whereas all 20 animals between 2 and 5 years of age died or needed treatment to survive. Young animals which needed no treatment to survive were refractive to challenge for at least 1 year after their last patent parasitemia. Older animals which required treatment to survive were also challenged at intervals after therapy. Three animals infected for 49 to 75 days before treatment were rechallenged 198 to 296 days later. Extensions in prepatent periods ranged from 5 to 13 days when compared to controls and the resulting infections were of a relapsing nature followed by self-cure. Effects of this disease on clinical parameters of previously infected animals were minimal. One animal infected for 196 days and rechallenged 501 days later had a prepatent period of 14 days as compared to 5 days for controls. This animal developed a brief relapsing infection followed by self-cure. Animals which were infected for periods of 41 to 77 days, received treatment, and were then rechallenged from 600 to 900 days later, showed some resistance to infection. Prepatent periods were extended from 1 to 3 days over those of control animals and although the resulting disease was severe, one of four animals self-cured without treatment. When animals which had self-cured primary challenges were rechallenged at periods up to 2 years later, they were completely refractory. When 12 animals which were presumed to be immune to syringe-passaged *T. congolense* were challenged by tsetse fly bite with the same strain of trypanosome, an appreciable immunity was evident. Five of twelve immune animals did not become patent while the other seven developed mild infections without severe clinical signs. All nine controls developed severe infections with eight requiring treatment to survive. When animals immune to the Trans-Mara I strain of *T. congolense* were challenged either by syringe or tsetse fly bite with a heterologous strain of *T. congolense* obtained from a different geographical area, no evidence of immunity was detected.

INDEX DESCRIPTORS: *Trypanosoma congolense;* Hemoflagellate; Protozoa, parasitic; Bovine host; Calf, Hereford; Immunity, natural and acquired.

INTRODUCTION

In the field, there is some evidence of natural and acquired immunity in cattle to trypanosomiasis. It has been postulated that young animals are more resistant to trypanosomiasis than adults (Fiennes 1970), possibly through transmission of an immune factor to calves born of immune or partially immune dams (Whiteside 1962). Certain breeds of cattle also appear to be naturally resistant to trypanosome infection Murray *et al.* (1979). Attempts to induce immunity to trypanosomiasis under field conditions, however, have produced contradictory results. Reports of several investigators have shown no evidence of im-

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munity in cattle maintained under therapy in endemic areas over long periods of time (Hornby 1941; Wilson *et al.* 1975). Other workers, however, claim that drug therapy induced a degree of protective immunity in treated animals (Bevan 1928; van Saceghem 1938; Fiennes 1953; Soltys 1955; Smith 1958; Wilson *et al.* 1976). Many of these field observations, however, are difficult to interpret because of the use of small numbers of animals of unknown age and condition, the question of persisting trypanocides, and the meager information concerning the antigenic nature of the trypanosome complex in given endemic areas.

In the laboratory a variety of immunization procedures have been used which produce a strong resistance in animals to a challenging trypanosome infection (Dodin and Fromentin 1962; Johnson *et al.* 1963; Seed and Weinman 1963; Duxbury and Sadun 1969; Wellde *et al.* 1973, 1979). None of these procedures, however, has been shown to be effective against the disease in nature. This has been due, in part, to the variant specific nature of the protective immune response and to the relatively obscure antigenic structure of the naturally transmitted metacyclic trypanosome. The early literature on the subject of immunity to African trypanosomiasis has been amply reviewed by Taliaferro (1929), and the more recent literature by Clarkson (1976) and Murray and Urquhart (1977).

The lack of substantial laboratory investigations regarding immunity in the bovine to *Trypanosoma congolense* led us to examine questions concerning immunity in reference to the following: age resistance, self-cure, chemotherapeutic cure, and the relationship between blood- and tsetse fly-induced infections.

Materials and Methods

Hosts. Cattle of a predominantly Hereford breed were obtained from the veterinary department farm at Kabete or from other trypanosomiasis-free areas in Kenya. Upon their arrival at our laboratory, all animals were routinely treated before experimentation with recommended levels of Terramycin (Pfizer International, New York, NY, USA), phenamidine (May and Baker, Dagenham, England), and Ranizole (Merck Sharpe and Dohme, B.V., Haarlem, Netherlands). Ranizole treatment was continued on a periodic basis. All animals also received foot and mouth vaccine (Wellcome, Kenya). In general, the experimental animals were kept outside and supplemental food was provided during periods of poor pasture conditions. All experimental animals were dipped or sprayed in an acaricide weekly, with the exception of animals undergoing tsetse fly challenge. Randomly bred, albino mice were used as recipients for subinoculated blood and for maintenance of trypanosomes.

Parasites. The Trans-Mara I strain of *Trypanosoma congolense* which was isolated from an infected cow in the Trans-Mara area near the Kenya–Tanzania border in 1966 was the primary parasite used in these studies. A stabilate was made from a pool of blood collected from three infected steers in 1971. Other stabilates were prepared in 1973 and 1975 and all animals in this study were infected with trypanosomes originating from one of these three stabilates. Usually, infected mice were used as donors after infection with stabilate trypanosomes. Sometimes, however, animals were infected or challenged with blood obtained from infected cattle.

For blood-induced infection or challenge, trypanosomes in heparinized blood were enumerated in a hemocytometer and diluted with phosphate-buffered saline (pH 7.8) containing 5% glucose and 10% fetal calf serum and injected into the jugular vein. Cattle were infected and challenged with 10,000 *T. congolense* per 500 lb body wt unless otherwise noted.

For tsetse fly-induced infection or challenge, newly emerged flies (*Glossina morsitans*) were fed on an infected bovine

donor for 14 consecutive days. Thereafter, the flies were fed for 5-day intervals on noninfected bovines until needed to induce a challenging infection.

A second strain of *T. congolense* was used for testing heterologous immunity. This parasite (designated Yoani 1 strain) was isolated in 1977 from an infected dairy cow at Yoani, Kenya, about 40 miles south of Nairobi.

Detection of parasites. All animals were tested for the presence of trypanosomes by injecting their blood (0.5 ml) into mice intraperitoneally before the initiation of experiments. Subinoculations of blood were also done in some experimental animals in an effort to detect subpatent infections.

Parasitemias in experimental animals were estimated by counting the numbers of trypanosomes per 100 leucocytes on thick blood smears and relating these values to the total leucocyte counts per cubic millimeter.

Chemotherapy. Curative chemotherapy was initiated with Berenil (Farbwerke Hoechst, Frankfurt (M), W. Germany) at a level of 1.05 g of active ingredient per 660 lb body wt. Generally, animals which were treated were severely anemic, extremely weak, and occasionally prostrate. These animals appeared to be near death at the time of treatment.

Assessment of immunity. Immunity was assessed by comparing experimental and control animals in terms of prepatent periods, levels and frequency of parasitemia, hematologic parameters, general clinical signs, and the ability to survive a challenging infection.

Hematology. Packed cell volumes (PCV) were determined by the microhematocrit method and leucocytes were counted using an electronic cell counter (Coulter Electronics, Harpenden, England). Methods used in collecting samples and counting thrombocytes have been published previously (Wellde *et al.* 1978). Reference in the text to experimental values are given plus or minus one standard deviation (±SD) unless otherwise noted.

Results

Effect of Dose of Parasites and Sex of Host

Within the range of numbers of trypanosomes injected into cattle, no relationship between dose and survival time of the animals was observed. The dose of trypanosomes was, however, related inversely to the prepatent period (Fig. 1). Both male and female animals developed similar infections and there was no apparent difference in survival times between animals of different sexes.

Age Resistance

Table 1 depicts the results of infections in animals of different age groups. It can readily be seen that most animals under 1 year of age survived the infection without treatment. Some animals between 1 and 2 years of age also were able to survive the infection, whereas all animals over 2 years of age either died or required treatment to survive.

Infections in Young Host Animals

Even though young animals were able to survive the infection without treatment, they underwent severe disease. Clinical parameters were compared over a 31-week period in 11 young animals who survived and 6 uninfected controls. Figure 2 shows

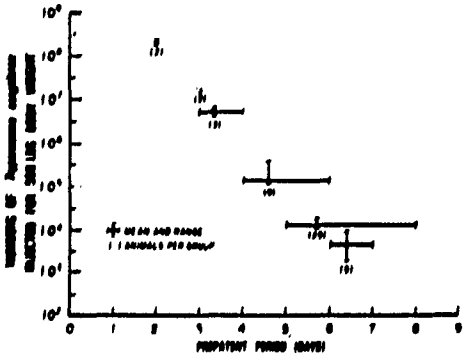

FIG. 1. The effect of numbers of *Trypanosoma congolense* injected on the prepatent period in bovines.

TABLE I
The Effect of Age on *Trypanosoma congolense* Infections in Cattle

Age range (years)	Number of animals	Median survival time[a] (weeks, days)	Range of survival times (weeks, days)	Number self-cures[b]
0.3–1	11	>78.0	7.1 to >78.0	10 (91)
>1–2	11	24.4	5.5 to >78.0	2 (11)
>2–3	11	11.5	5.5 to 30.6	0 (0)
>3–4	5	6.3	6.1 to 13.6	0 (0)
>4–5	2	7.1	4.2 to 9.0	0 (0)
>5–6	2	8.1	8.0 to 8.3	0 (0)

[a] Based on time to treatment or day of death.
[b] Percentage shown in parentheses.

the average level of trypanosomes in the peripheral blood of the survivors over a 31-week period. Average levels of parasitemia were gradually reduced as the disease progressed. Animals had patent infections throughout the first 8 weeks; thereafter an increasing number of animals became apatent for periods which increased with time.

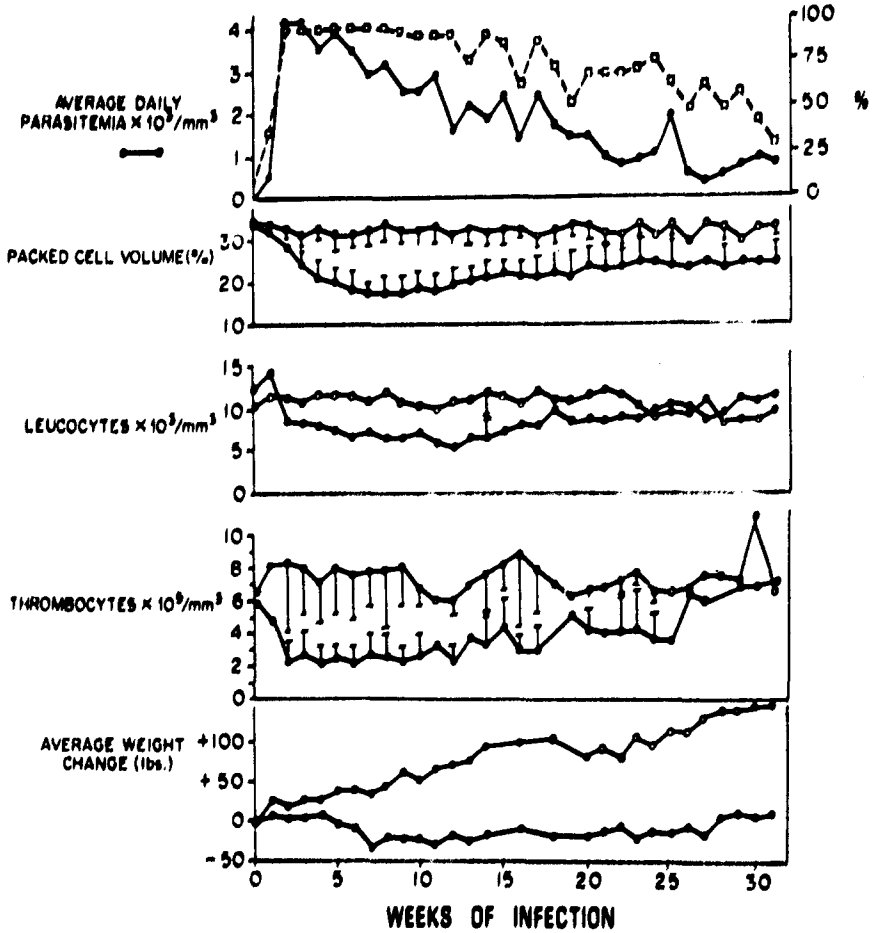

FIG. 2. Clinical parameters of young animals surviving *Trypanosoma congolense* infections. Data points where ± 1 SD overlap have not been plotted. ●, Infected; ○, control; □, thick blood smears positive (%).

An average preinfection packed cell volume of 34.0(±4.0) was reduced to a level of 17.9(±3.2) at 8 weeks after infection (Fig. 2). Packed cell volumes gradually increased after this time and by 31 weeks after infection had risen to 24.1(±4.8). Packed cell volumes did not appear to reach preinfection levels in individual animals for long periods even though trypanosomes were found infrequently in the blood. Thrombocytopenia and leucopenia also were prominent manifestations of the disease (Fig. 2). Intermittent fever was accompanied by an early weight loss after which a minimal weight gain was apparent. Controls of the same age, however, had gained an average of 116 lb while the infected animals gained only an average of 9 lb during the 31-week period (Fig. 2). Many of these young infected animals remained small in stature throughout their adult life (Fig. 3).

Immunity in Self-Cured Calves

Animals which had apparently self-cured the primary infection and whose blood was negative when subinoculated into mice were challenged with the same strain of *Trypanosoma congolense* up to a year after their last patent parasitemia. No detectable infections developed in the self-cured animals whereas the controls developed typical infections and required treatment to survive (Table 2).

Infections in Adult Host Animals

Animals over 2 years of age developed an acute or chronic course of disease that required treatment or was fatal. Clinical parameters were compared in 11 adults and 11 uninfected controls (Fig. 4). The average parasitemia in adult animals was twice that of the surviving young animals and the anemia which developed was usually somewhat more severe than that of young animals. A leucopenia, which was comparable to that found in young animals, was also present (Fig. 4). Average thrombocyte levels were lower in infected adult animals than in young animals. Weight loss was severe in adult animals with up to a 34% decrease in preinfection values. Ten of the eleven infected animals died or required treatment to survive by Week 15 of infection. The remaining infected animal developed a protracted chronic course of disease and died during Week 32 of infection. This chronic disease state was characterized by a low-level relapsing parasitemia accompanying a continued low PCV.

Immunity in Treated Adult Hosts

Adult animals which required therapy to survive were challenged along with controls at a later time. Table 3 shows that an appreciable immunity had developed in these animals and many self-cured the challenge

FIG. 3. An example of the stunting effect of *Trypanosoma congolense* infection on young animals can be seen in the animal in the foreground. The control is in the background. At the time of infection, 13 months previously, both animals were of similar age, weight, and stature.

TABLE II
Results of Primary Challenge of Previously Infected, Self-Cured Bovine Host

Animal No.	Initial infection with *Trypanosoma congolense*						Primary challenge (1 × 10⁶/300 lb)		
	Age (years)	Sex[a]	Dose per 300 lb	PP[b] (days)	Last patent parasitemia (weeks, days)	Interval[c] (weeks, days)	Age	PP[d] (days)	Results[e] (weeks, days)
1	0.5	M	2.8×10^6	8	54.4	25.0	2.0	NP	NDI
2	1.3	MC	1.0×10^6	5	61.1	31.6	3.0	NP	NDI
3	0.3	F	1.0×10^6	5	56.2	36.5	2.0	NP	NDI
4	0.5	M	2.9×10^6	6	30.5	48.5	3.1	NP	NDI
5	1.4	MC	1.0×10^6	5	38.2	54.5	3.1	NP	NDI
									T (0.3)[f]

[a] F, Female; MC, castrated male; M, male.
[b] PP, prepatent period.
[c] Time between last patent parasitemia and challenge.
[d] PP, prepatent period; NP, not patent.
[e] NDI, no detectable infection.
[f] Average of three control animals for primary challenge.

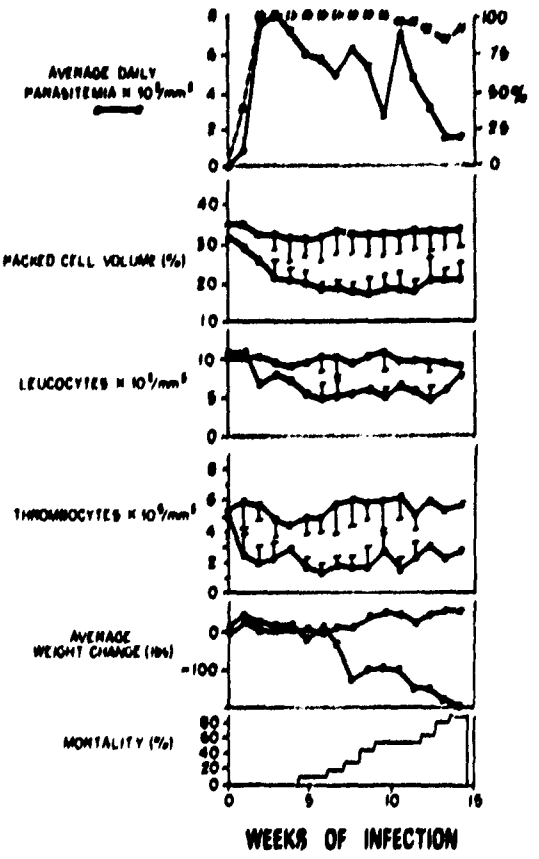

FIG. 4. Clinical parameters of adult animals which either required treatment to survive or died from *Trypanosoma congolense* infection. Data points where ± 1 SD overlap have not been plotted. ●, Infected; ○, control; □, thick blood smears positive (%).

infection. Even when the challenging infection was given about 2 years after treatment there was evidence of persisting immunity. Although most of these animals challenged at this time needed treatment to survive, their infections were of a chronic nature and less severe than those of control animals. Treatment was required in these animals at a later time than in their primary infections or in the controls. Figure 5 illustrates the pattern of parasitemia and level of packed cell volume in an animal undergoing infection, treatment, and challenge. The challenging infection was much less severe than the primary infection; the animal had limited periods of parasitemia which were

TABLE III
Results of Primary Challenge of Previously Infected and Treated Cattle Hosts

Initial infection with *Trypanosoma congolense*							Primary challenge (1 × 10^4 500 lb)		
Animal No.	Age (years)	Sex[a]	Dose per 500 lb	PP[b] (days)	Time to treatment (weeks, days)	Interval[c] (weeks, days)	Age (years)	PP (days)	Results[d] (weeks, days)
6	1.0	F	6.8×10^6	3	7,0	28,6	1.7	14	SC (17,0)
7	4.4	F	1.0×10^4	6	9,0	30,0	5.2	10	SC (11,5)
8	2.7	F	1.0×10^4	5	11,5	42,2	3.8	18	SC (15,5)
9	2.6	F	1.0×10^4	5	6,6	47,1	3.7	13	T (36,6)
10	1.9	F	1.3×10^4	5	28,0	71,4	3.9	14	SC (4,4)
11	1.6	MC	8.4×10^3	6	5,5	86,0	3.4	8	T (21,3)
12	1.9	MC	1.0×10^4	6	5,5	86,0	3.7	6	T (11,5)
13	2.3	MC	1.9×10^4	5	11,0	122,5	4.9	6	T (27,0)
14	3.4	F	1.3×10^4	5	5,1	128,4	6.0	6	SC (29,2)
							4.1	5.5	T (9,4)[e]

[a] F, Female; MC, castrated male.
[b] Prepatent period.
[c] Time between treatment and challenge.
[d] SC, self-cure (time of last patent parasitemia after challenge); T, treated (time since challenge).
[e] Average of eight control animals for primary challenge.

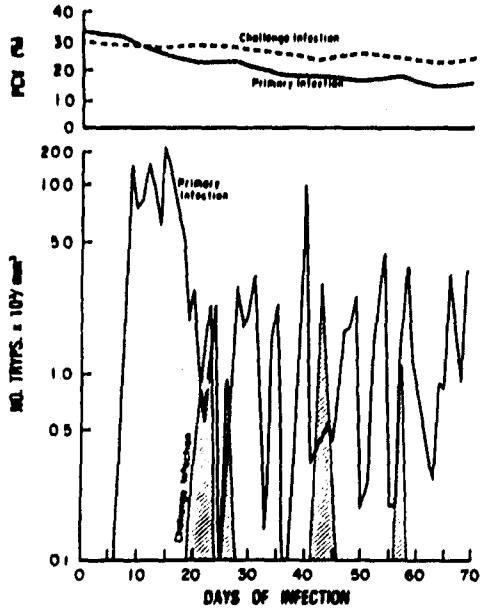

FIG. 5. Parasitemias and packed cell volumes of an animal treated 82 days after primary *Trypanosoma congolense* infection and challenge 296 days later.

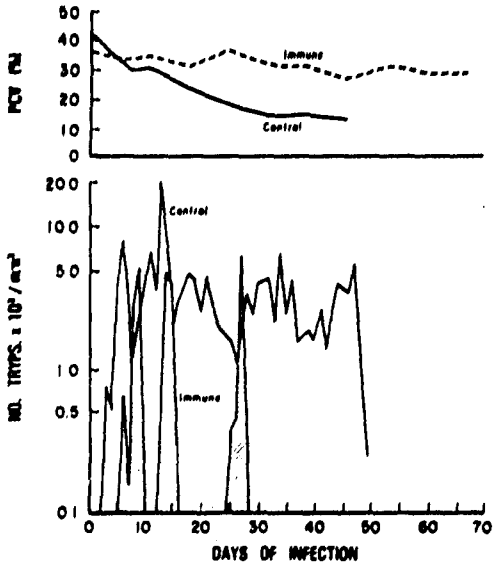

FIG. 6. Parasitemias and packed cell volumes of an immune and control animal challenged with 1×10^7 *Trypanosoma congolense* from a relapse parasitemia obtained from a chronically infected bovine.

similar to that of a chronically infected animal and a minimal decrease in packed cell volume and other hematologic parameters.

When animals described in Table 3 were challenged a second time, no detectable infections or clinical signs of disease were observed while all controls developed parasitemia and required treatment to survive (Table 4). Animals also were strongly resistant to challenge with relapse parasites obtained from chronically infected bovines. Figure 6 illustrates the pattern of parasitemia and level of packed cell volume in an animal immunized by infection and cure with those of a control. Both animals were challenged with parasites isolated from a bovine undergoing a relapsing infection of 250 days duration. When compared to the control animal, the infection in the immunized animal was brief and much less severe. The control required treatment while the immunized animal self-cured.

Tsetse Fly Challenge

Animals immune to challenge with syringe-passaged trypanosomes were exposed to tsetse fly challenge with the ho-

TABLE IV

Results of the Second Challenge of Bovines Requiring Treatment or Self-Cured after Primary Challenge with *Trypanosoma congolense*

Group	Number of animals	Interval[a] (months)	Prepatent[b] period (days)	Result[c] (weeks, days)
Self-cure	3	5–30	NP	NDI
Treated	3	6–10	NP	NDI
Control (Average)	6	—	5.5	T (8,0)

[a] From last patent parasitemia or treatment.
[b] NP, not patent.
[c] NDI, no detectable infection; T, treated (time since challenge).

TABLE V
Results of Tsetse Fly Challenge of Animals Immunized against Blood Forms of *Trypanosoma congolense*

Group	Age[a] (years)	Number patent/ number challenged	Prepatent period[b] (days)	Days of patent infection[c,b]	Lowest PCV[c,d]	Result[e]		
						NDI	SC	T
Immune[f]	4.6(1.9–8.0)	7/12	20.1(6–56)	6.4(0–32)	29.1(25–34)	5	7	0
Partially immune[f]	5.4(4.2–6.3)	3/3	15.3(13–19)	79.7(61–100)	20.3(18.5–22.5)	0	2	1
Control	3.5(1.2–7.4)	9/9	10.8(10–13)	96.6(83–100)	17.3(15.5–19.5)	0	1	8

[a] Range is given in parentheses.
[b] First 100 days after Day 10.
[c] Packed cell volume.
[d] NDI, no detectable infection; SC, self-cure; T, required treatment.
[e] Six animals which self-cured the primary infection and six animals immunized by repeated infection and drug cure.
[f] Animals having a single infection and treatment.

mologous strain of *T. congolense*. Each of 12 immune, 3 partially immune, and 9 control animals received an average of 428 fly bites from a pool of flies having a 32% infection rate of metacyclic trypanosomes. Of the 12 immune bovines challenged by fly bite, 5 did not develop parasitemia or clinical evidence of disease (Table 5). The other 7 had limited periods of patent parasitemia (Fig. 7), and only 1 animal developed signs of clinical disease. All 12 immune animals survived without treatment while all 9 control animals developed severe infections and 8 required treatment.

Average parasitemias were greatly reduced in immune animals and followed a relapsing pattern similar to that of chronic infections or that of immune animals challenged with blood forms. Prepatent periods were not always increased in immune animals, however, and 3 immune animals had prepatent periods similar or shorter than controls. Although parasites appeared in the blood of these animals early after challenge, they were suppressed quickly (Fig. 8). Clinical parameters such as PCV (Fig. 9), thrombocyte levels (Fig. 10), and leucocyte levels (Fig. 11) remained within normal limits in immunized animals while the values in controls were severely affected. These results were confirmed in two separate experiments performed 1 and 2 years later, respectively.

One animal, which had undergone primary infection and challenge in 1970 was rechallenged periodically during the sub-

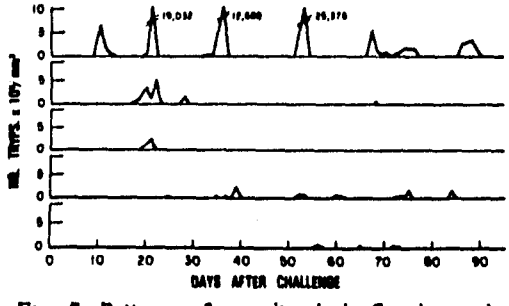

FIG. 7. Patterns of parasitemia in five immunized bovines after tsetse fly challenge with *Trypanosoma congolense*.

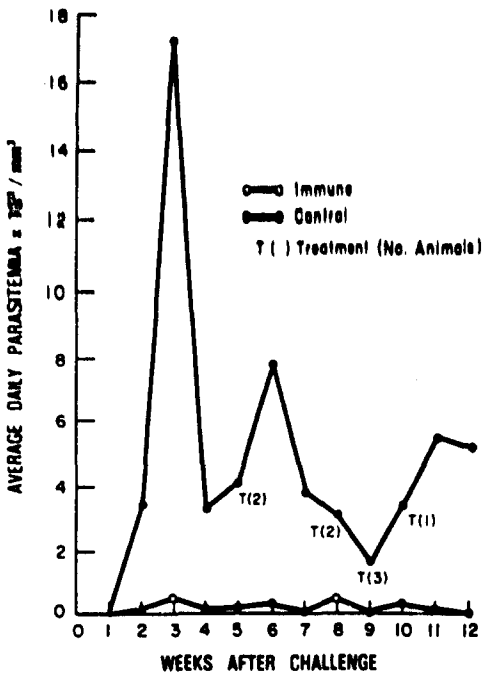

FIG. 8. Average parasitemia levels of immunized and control bovines after tsetse fly challenge with *Trypanosoma congolense*.

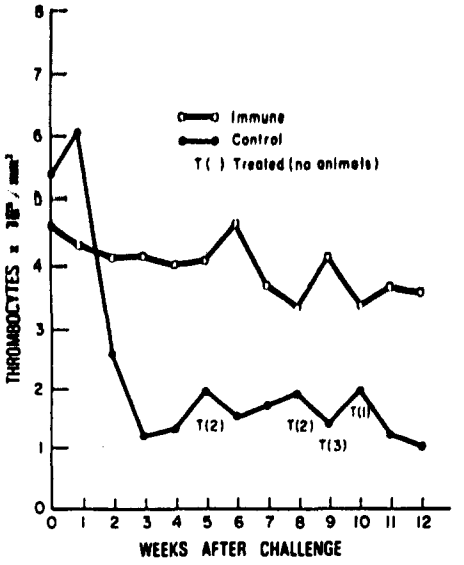

FIG. 10. Average thrombocyte levels of immunized and control bovines after tsetse fly challenge with *Trypanosoma congolense*.

sequent 6 years with syringe-induced infections. This animal was challenged by tsetse fly bite in 1977. Table 6 summarizes the results over the 7-year period. Control animals injected at each challenge either required treatment to survive or died.

Heterologous Challenge

To determine whether or not cross-strain immunity was present in animals immune to

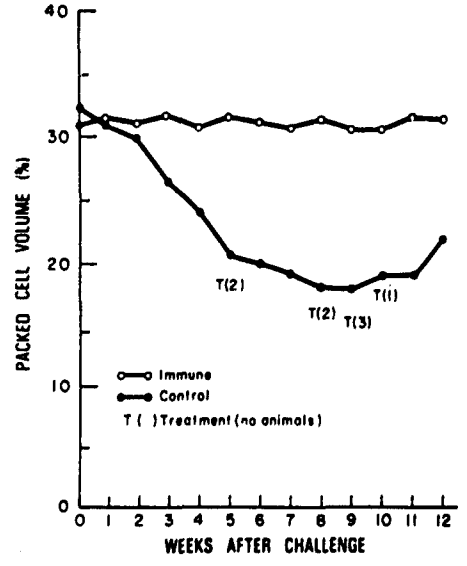

FIG. 9. Average packed cell volumes of immunized and control bovines after tsetse fly challenge with *Trypanosoma congolense*.

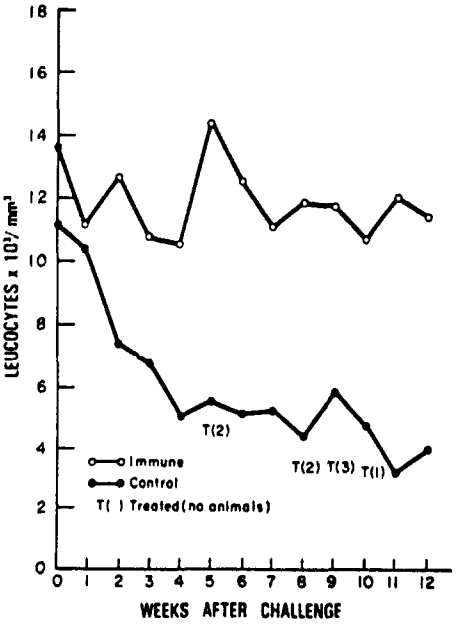

FIG. 11. Average leucocyte levels of immunized and control bovines after tsetse fly challenge with *Trypanosoma congolense*.

TABLE VI
History of Host Animal 151

Procedure	Date	Source[a]	Infection method[b]	Dose/ 500 lb	Prepatent period[c] (days)	Result[d]	Lowest PCV[e] (%)
Primary infection[f]	14-9-70	B	S	1.0×10^5	5 (5)	T (196)	11.0
Challenge							
1	11-8-72	M	S	1.0×10^4	14 (5)	SC (32)	31.5
2	5-3-75	M	S	1.0×10^4	NP (5)	NDI	31.0
3	10-7-75	B (Relapse)	S	1.0×10^7	5 (3)	SC (34)	27.0
4	17-3-76	M	S	1.0×10^4	NP (5)	NDI	30.0
5	15-12-76	M	S	1.0×10^4	NP (5)	NDI	29.0
6	26-9-77	B	T	?	19 (11)	SC (21)	33.0

[a] B, Bovine; M, mouse.
[b] S, Syringe; T, tsetse fly.
[c] NP, not patent; values for controls shown in parentheses.
[d] T, treated; SC, self-cure (last patent parasitemia); NDI, no detectable infection. Days since challenge shown in parentheses.
[e] PCV, packed cell volume.
[f] With *Trypanosoma congolense*.

the Trans Mara-I strain of *T. congolense*, three immune and three control animals were challenged with the Yoani strain of *T. congolense* by blood-induced or tsetse fly-induced infections (Table 7). No immunity was observed in any of the animals challenged by either method. All animals were treated during Week 5 of infection when packed cell volumes had decreased to below 20%.

Discussion

Our studies demonstrated that under certain conditions an appreciable immunity to *Trypanosoma congolense* can develop in bovines. We found a substantial age resistance to *T. congolense* and although young animals underwent a relatively severe disease process, almost all survived while animals over 2 years of age invariably succumbed to infection. Although the mechanism(s) for such resistance is not clear, in our experiments it did not involve specific maternal antibody since the dams of our calves had never been infected and the calves had been weaned at least 1 month before infection. These studies confirm and

TABLE VII
Results of Blood- and Tsetse Fly-induced Challenge of Host Animals Immune to the Trans-Mara I Strain of *Trypanosoma congolense* with a Heterologous Strain (Yoani-I)

Group	Type challenge	Number patent/ number challenged	Prepatent period[a] (days)	Result[b]		
				NDI	SC	T
Immune	Blood	3/3	7.3(6–9)	0	0	3
Control	Blood	3/3	7.0(6–8)	0	0	3
Immune	Tsetse fly	3/3	13.6(12–15)	0	0	3
Control	Tsetse fly	3/3	13.6(13–14)	0	0	3

[a] Range given in parentheses.
[b] NDI, no detectable infection; SC, self-cure; T, required treatment.

extend the observations of others (reviewed by Fiennes 1970).

Although Weitz (1970) suggested there was no evidence for an acquired protection in animals after recovery from the disease, we have shown that young surviving animals are resistant for extended periods of time to a challenge infection of the same strain by either syringe inoculation of blood forms or by tsetse fly bite (metacyclic forms). Many of these immune animals, however, are stunted and are relatively nonproductive. As well as being a poor source of meat, the small stature of females infected early in life may lead to problems in calving. We have observed the death of one of our self-cured experimental animals due to the inability to complete parturition because of her small pelvic diameter.

Animals undergoing infection and berenil treatment also showed resistance upon syringe- or tsetse-challenge with the same strain. Most of these animals self-cured the first or second challenge infections. Premunity, (resistance associated with concurrent infection) did not play a role in this protection since the animals had been given curative therapy to terminate the primary infection. The resistance appeared to be associated with the duration of infection, the time elapsing between treatment and challenge (Table 3), and the number of times the animal has been infected (Table 4). The short period that effective levels of berenil persist in the blood of the bovine precludes any complicating drug effect in these studies. Trials in our laboratory showed that berenil (7 mg/kg) had an effect on infectivity for 12 days and on the prepatent period for up to 18 days but not at 25 days or longer after injection (unpublished data). This is in agreement with previously published work (Cunningham *et al.* 1964).

While we have shown that a substantial immunity can be induced experimentally by infection and cure, the reasons are not well understood why it has not been more apparent in nature. Since most of the failures to produce an immunity in animals in the field by this method have been in areas of high tsetse challenge, we believe that the interval between treatment and reinfection is important. It is known that the lymphoid system in *T. congolense*-infected bovines undergoes atrophy and depletion of lymphocytes (Kaliner 1974; Murray 1974). Morrison and Murray (1978) have shown a marked depletion of immunoglobulin-containing cells in the spleens of *T. congolense*-infected mice and these findings are consistent with the reports of deficient immunologic responses to a variety of antigens in *T. congolense*-infected hosts (Mansfield and Wallace 1974; Holmes *et al.* 1974). Thus the response to the trypanosome by the infected host may also be defective and could account, in part, for the parasites' survival (Murray and Urquhart 1977). Little is known about the repopulation and recovery of the lymphoid system of the infected bovine after treatment, but reinfection soon after therapy may find the animal with reduced immunological competence. In our experiments, animals were given relatively long periods to recover after treatment and under these circumstances the secondary immunological response was surprisingly efficient in controlling and eradicating the parasitemia (Table 3 and Fig. 5).

It also appears that the antigenic composition of populations of *T. congolense* in nature is complex (Dar *et al.* 1973; Wilson *et al.* 1973) and the presence of different serodemes of *T. congolense* as well as those of other species of trypanosomes in a given area would likely complicate the acquisition of immunity. Barbet and McGuire (1978) have identified similar cross-reacting groups in surface antigens from clones of *T. brucei* and *T. congolense*. It seems unlikely, however, that these groups elicit a broad spectrum protective response given the extremely specific nature of immunity to trypanosome infections. We detected no cross-immunity either against blood or

tsetse fly induced infections with an isolate of *T. congolense* from a different area in Kenya.

Our studies indicate that there is probably a limitation imposed by the parasite genome on the occurrence of different antigenic types which arise in infected animals. The progressively decreasing levels of parasitemia and increasing periods of apatency associated with self-cure suggest that either the host response is increasingly efficient or that the parasites' capability to produce different antigenic variants eventually becomes exhausted. The fact that animals become immune is also not consistent with a process which requires the generation of antigenic variants through mutation.

Blood-induced infections immunized animals against subsequent tsetse fly challenge with the homologous trypanosome. Since 5 of 12 immunized animals did not develop patent parasitemias it appeared that immunity had an early influence on the infection. Whether this was directed at metacyclic trypanosomes soon after inoculation by the fly or later against the resulting tryptomastigotes remains to be determined. It is possible that similar surface antigens exist among metacyclic and tryptomastigote forms of the same trypanosome serodeme although it seems more likely that immunity was directed against the earliest tryptomastigotes. While infections became established in the other immunized animals, the relatively brief periods of low-level parasitemia which occurred in the absence of clinical signs indicate that a substantial immunity was directed against the surviving tryptomastigotes. From these findings, it seems that similar antigenic types occurred early after syringe or tsetse fly passage of *Trypanosoma congolense* of the same serodeme. This supports Gray's (1965) contention that a relatively predictable series of antigenic types appears early during the course of blood- or tsetse fly-induced infections.

At the present time it is difficult to reconcile this apparently regulated system with recent evidence indicating a complex antigenic heterogeneity in metacyclic trypanosome populations (LeRay *et al.* 1977).

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